Environmental Health Perspectives
Vol. 30, pp. 179–184, 1979

Evaluation of Feasibility of Mutagenic Testing of Shale Oil Products and Effluents

by J. L. Epler,* T. K. Rao,* and M. R. Guerin†

In an effort to gather preliminary information on the potential genetic hazards of proposed or existing oil shale technologies, we have begun a correlated analytical and genetic analysis of a number of test materials. The work is divided into two phases: one deals with known compounds expected to occur in the environment through shale oil production or use; the other deals with actual samples from existing or experimental processes. A fractionation procedure has been applied to crude product and aqueous product material from an oil shale process. Mutagenicity of the various fractions was assayed by using reversion of histidine-requiring auxotrophs of *Salmonella typhimurium* (strain TA100, base-substitution mutant; TA98 and TA1537, frameshift mutants). In order to incorporate metabolic activation of these fractions and compounds, we used liver homogenates (S-9) from rats induced with Aroclor 1254 in the standard plate assay. Preliminary results implicate chemicals occurring in the basic (ether-soluble) and the neutral fractions as potential genetic hazards. Chemical constituents of these fractions (identified or predicted) were tested individually for their mutagenic activity and correlated with the genetic monitoring.

Introduction

A major concern with the development of the oil shale technology is the potential for long-term health hazards such as carcinogenesis, mutagenesis, and teratogenesis. The exposures of workers to crude shale oils, contaminated aqueous materials, particulate matter, and air pollutants represent potential hazards. Materials leached from both raw shale and spent shale are additional routes for human exposure. Adverse human health effects might result, particularly from long-term exposure to polycyclic aromatic hydrocarbons and trace elements emitted in various forms from the shale oil or synthetic fuel plant (*1*).

In order to rapidly and inexpensively ascertain the potential mutagenicity hazards of various test materials, we have examined the feasibility of using short-term genetic assays to predict and, in some cases, aid in isolating and identifying chemical mutagens. Furthermore, recent studies (*2*) have shown that there is an extremely high correlation between the ability of a compound to induce genetic damage and the carcinogenic potential of the compound. Thus, the mutagenicity assay might act as a prescreen for carcinogens.

In the studies presented here, we have used the Ames *Salmonella* histidine-reversion system (*3*) to assay the mutagenic potential of crude shale oil, natural crude oil, and the product water from a shale oil process. Mutagenicity data on isolated or suspected organic components are also presented. The results support the use of the short-term genetic tests in examining crude mixtures and point to the advantages of coupling the bioassays with chemical fractionation. Preliminary results (*4, 5*) have previously been reported, and comparative studies with synthetic fuels from the liquefaction of coal have been carried out (*6–8*).

Materials and Methods

Samples

Samples and their sources were: (1) a composite natural crude oil "control" sample obtained through the courtesy of Dr. Dee Latham of the

* Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.
† Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

Laramie Energy Research Center [composite of the following oils: Prudhoe Bay 30%; Wilmington, California 20%; South Swain Hills, Alberta 20%; Gach Saran, Iran 20%; Louisiana-Mississippi Sweet 10%; and ABL (Arabian) 10%]; (2) a crude shale oil sample from the above-ground simulated *in situ* oil shale retorting process; and (3) the aqueous product water consisting of the centrifuged water of combustion from the same process (both samples 2 and 3 courtesy of Dr. Richard Poulson of the Laramie Energy Research Center). We recognize the possibility that these samples may bear no relationship to the process as it may exist in the future, nor should it be construed that these materials are representative of all natural crudes and shale oil processes. They are used here simply as appropriate and available materials for the feasibility study.

Fractionation

The fractionation scheme described by Swain et al. (*9*), as modified by Bell et al. (*10*), has been applied to the product from the oil shale process, the composite natural crude, and aqueous samples. The separation scheme has been illustrated and detailed elsewhere (*6*). Subfractionation of the neutral portion was accomplished with a Florisil column. Reagent-grade solvents and glass-distilled water were used for extraction and chromatography.

Mutagenicity Testing

The *Salmonella typhimurium* strains used in the various assays were: TA1535 *hisG46, uvrB, rfa* (missense); TA100 *hisG46, uvrB, rfa* (missense plus R factor); TA1537 *hisC3076, uvrB, rfa* (frameshift); TA1538 *hisD3052, uvrB, rfa* (frameshift); TA98 *hisD3052, uvrB, rfa* (frameshift plus R factor). All strains were obtained through the courtesy of Dr. Bruce Ames, Berkeley, California.

In the routine screening of fractionated materials, the two strains TA98 and TA100 were generally used. Experimental procedures have been given by Ames et al. (*3*). The strain to be treated with the potential mutagen(s) is added to soft agar containing a low level of histidine and biotin along with varying amounts of the test substance. The suspension containing approximately 2×10^8 bacteria is overlaid on minimal agar plates, and revertants to wild type are counted after a 2-day incubation. The assay is quantitated with respect to dose (added amount) of test material and modified to include treatment with the liver homogenate required to metabolically activate many compounds.

Fractions and/or control compounds to be tested were suspended in dimethylsulfoxide (DMSO, supplied sterile, spectrophotometric grade from Schwarz/Mann) to concentrations in the range 10–20 mg/ml solids. The potential mutagen was in some cases assayed for general toxicity (bacterial survival) with strain TA1537. Generally, the fraction was tested with the plate assay over at least a 1000-fold concentration range with the two tester strains TA98 and TA100. Revertant colonies were counted after 48 hr incubation. Data were recorded and plotted versus added concentration, and the slopes of the induction curves were determined. Positive or questionable results were retested with a narrower range of concentrations. All studies were carried out with parallel series of plates plus and minus the rat liver enzyme preparation (*3*) for metabolic activation. Routine controls demonstrating the sterility of samples, enzyme or rat liver S-9 preparations, and reagents were included. Positive controls with known mutagens were also studied in order to recheck strain response and enzyme preparations. All solvents used were nonmutagenic in the bacterial test system.

Results and Discussion

Oil Samples

In the investigation of the feasibility of the coupled analytical—mutagenicity assay approach, we fractionated the simulated *in situ* retorting shale oil sample and the composite natural crude "control" oil sample into primary acidic, basic, and neutral components. Each primary fraction was then assayed with the Ames strains; subsequently, the neutral portion was subfractionated and the subfractions were assayed for mutagenicity. The distribution by weight of the test materials, the "specific activity" (revertants/mg) of each fraction, and the contribution of each fraction to the mutagenic potential of the starting material (product of weight percent and specific activity) are listed in Table 1. Data are given for the frameshift strain TA98 with metabolic activation with enzyme preparations from Aroclor 1254-induced rats. The shale oil and natural crude samples both contain significant activity in the neutral fractions and, overall, appear to represent comparable mutagenic hazards. However, the shale oil material appears to contain additional activity in other fractions, particularly the B_E ether-soluble fraction. Note that the sum of activities from the neutral subfractions corresponds to the value obtained from the unfractionated neutral material.

Figure 1 shows the dose-response curves for two of the shale oil fractions. The slope of the linear portion of the induction curve represents the re-

Table 1. Distribution of mutagenic activity in fractions of natural crude and shale oils.[a]

No.	Fraction[d]	Composite crude[b] Relative weight, % of total	Composite crude[b] Specific activity, rev/mg[e]	Composite crude[b] Weighted activity, rev/mg[f]	Shale oil[c] Relative weight, % of total	Shale oil[c] Specific activity, rev/mg[e]	Shale oil[c] Weighted activity, rev/mg[f]
1	$NaOH_I$	1.3	0	—	1.02	256	3
2	WA_I	0.1	0	—	0.05	185	>1
3	WA_E	0.3	225	1	1.23	52	1
4	SA_I	0.2	0	—	0.09	0	—
5	SA_E	0.4	17	<1	0.26	159	>1
6	SA_W	0.1	750	1	0.55	160	1
7	B_{Ia}	1.3	8	1	0.20	1377	3
8	B_{Ib}	0.3	0	—	0.26	800	2
9	B_E	0.2	500	1	7.11	952	68
10	B_W	0.5	475	2	0.28	223	1
	Neutral	84.2	166 (200)[g]	140	86.66	112 (109)[g]	97
	Total	88.9		147	97.71		178
	Neutral subfractions						
11	Hexane						
	A	71.1	110	78	58.69	40	23
	B	—	—	—	2.14	625	13
	C	—	—	—	1.27	750	10
12	Hexane/benzene						
	A	7.8	350	27	4.38	238	10
	B	—	—	—	1.89	340	6
	C	—	—	—	1.39	320	4
13	Benzene/ether						
	A	8.9	583	52	12.43	65	8
	B	—	—	—	2.19	142	3
	C	—	—	—	1.29	253	3
14	Methanol						
	A	4.7	200	9	15.12	179	27
	B	—	—	—	0.49	684	3
	C	—	—	—	0.93	263	2
	Subtotal	92.5		166	102.21		112

[a] All assays carried out in the presence of crude liver S-9 from rats induced with Aroclor 1254.
[b] Initial sample weight, 24.501 g; chromatographed, 10.226 g.
[c] Initial sample weight, 24.042 g; chromatographed, 10.131 g.
[d] I = insoluble (fractions a and b), E = ether-soluble, W = water-soluble, WA = weak acid, SA = strong acid, and B = base.
[e] rev/mg = revertants/milligram, the number of histidine revertants from *Salmonella* strain TA98 by use of the plate assay with 2×10^8 bacteria per plate. Values are derived from the slope of the induction curve. NT = not tested.
[f] Weighted activity of each fraction relative to the starting material is the product of columns one and two. The sum of these products is given as a measure of the total mutagenic potential of each material. The value for the neutral fraction was calculated from the value for the weighted subfractions.
[g] Activity based on assay of the total neutral fraction before chromatography rather than on the summation of the individual subfraction.

vertants per milligram of the fraction (specific activity). Figure 2 represents the distribution of activities and material throughout the neutral subfractionation by column chromatography. Histograms such as this one aid in correlating the mutagenicity data with known or predicted constituents.

Comparable evaluations of crude synthetic fuels from coal liquefaction processes have pointed to consistently higher mutagenic potentials in synthetic fuels than in the materials assayed here (*4–6*).

Aqueous Sample

In order to extend the techniques to an aqueous material that might have more environmental importance, we assayed the centrifuged product water from the above-ground *in situ* retorting process (Table 2). Although a number of highly active materials occur, again in the basic fractions, the overall contribution of the contaminating organic portion appears to be low. Note also that the neutral portion, usually comprised of water-insoluble polyaromatic hydrocarbons, contains little mutagenic activity in this aqueous sample.

Mutagenicity of Organic Components

Chemical analyses of synthetic fuel materials (*11, 12*) and predictions based on work with tobacco smoke condensates (*9, 13, 14*) give the investigator

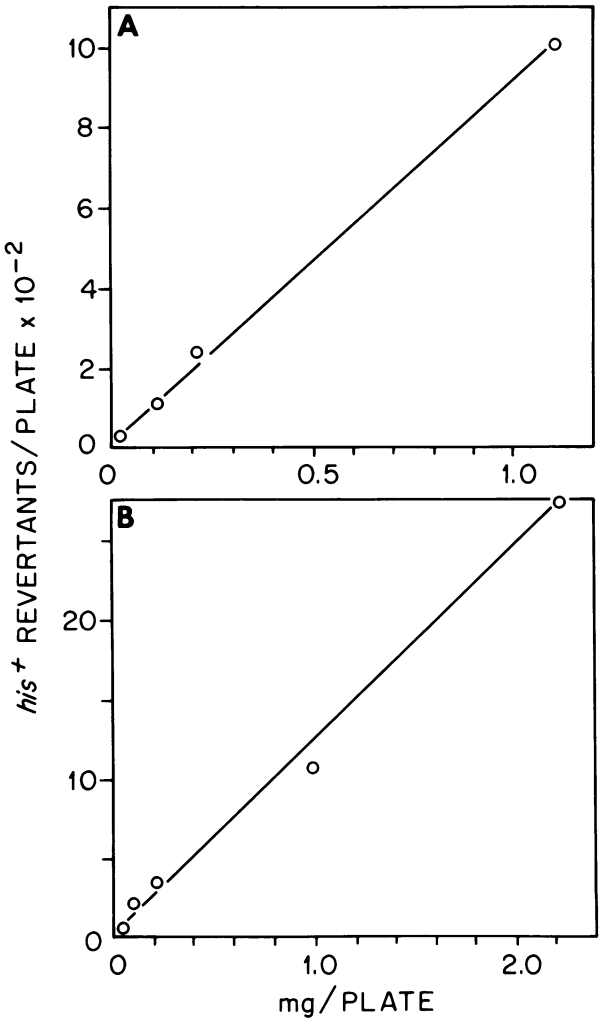

FIGURE 1. Induction of revertants in *Salmonella* strain TA98 with increasing concentration of (A) fraction 9, basic, ether-soluble from shale oil; and (B) fraction 9, basic, ether-soluble from product water with activation with an enzyme (S-9) prepared from rat livers induced with Aroclor 1254.

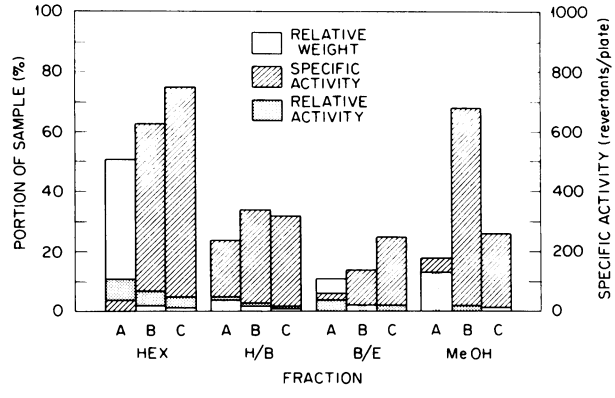

FIGURE 2. Histogram showing distribution of activity in neutral fractions of shale oil (above-ground simulated *in situ* retorting process).

Table 2. Distribution of mutagenic activity in fractions of aqueous sample.[a]

Fraction	Shale oil product water		
	Relative weight, % of total[b]	Specific activity, rev/mg	Weighted activity, rev/mg
$NaOH_I$	—	—	—
WA_I	1.5	397	5
WA_E	6.3	105	7
SA_I	3.9	0	—
SA_E	16.8	0	—
SA_W	65.0	0	—
B_{Ia}	0.1	52	<1
B_{Ib}	0.1	1468	1
B_E	2.7	1575	42
B_W	1.3	868	12
Neutral	2.4	52	1
Total			68

[a] 6 liters, continuous extraction, not filtered prior to extraction; initial sample = 10.1884 g.
[b] 1% (w/v) of test material.

a general view of the organic components of the various fractions. Based on these predictions, we list a selected group (Table 3) of organic compounds pertinent to shale oil and the preliminary results on mutagenicity in the *Salmonella* histidine-reversion system. Although a number of the compounds may be promoters or modifiers of carcinogenesis (that is, active in co-carcinogenesis, perhaps in some cases as inhibitors), they do not appear to be mutagenic.

The major mutagenic components appear to be heterocyclic nitrogen compounds, aromatic amines, and polycyclic aromatic hydrocarbons.

Utility of Short-Term Tests for Mutagenicity

The use of short-term tests for mutagenicity coupled with chemical fractionation and analyses of test materials appears to be a valid research approach. Their utility in predicting potential genetic hazard is obvious. However, we are attempting to verify the significance of the Salmonella data by extending a selected suite of synthetic fuel samples to other genetic assays (*16, 17*) including higher organisms. The use of the mutagenicity data as a prescreen for carcinogenesis may also be of value, but probably not in a quantitative sense. Too many factors modify the whole-animal carcinogenesis response to expect the type of mutagenicity screening used here to directly reflect the *extent* of carcinogenic potential.

Specific criticisms of the chemical extraction procedure and the biology can be levied: artifacts may be generated by the extreme conditions used in the Stedman fractionation (*9*); compounds could be destroyed or volatilized. Current research efforts are moving to milder techniques. Detection of

Table 3. Mutagenicity of chemicals identified in shale oil using *Salmonella* tester strains.[a]

Chemical name	CAS Registry No.	Strain	Mutagenicity
Phenol and aniline derivatives			
Phenol	108-95-2	98, 100	—
Phenol, dimethyl (xylenol)	1300-71-6	98, 100	—
2,3-	526-75-0	98, 100	—
2,4-	105-67-9	98, 100	—
2,5-	95-87-4	98, 100	—
2,6-	576-26-1	98, 100	—
3,4-	95-65-8	98, 100	—
3,5-	108-68-9	98, 100	—
Phenol, ethyl	25429-37-2	98, 100	—
2-	90-00-6	98, 100	—
4-	123-07-9	98, 100	—
Phenol, 2,4,6-trimethyl (mesitol)	527-60-6	98, 100	—
Aniline	62-53-3	98, 100	—
Aniline, dimethyl (xylidine)			
2,3-	87-59-2	98, 100	—
2,4-	95-68-1	98, 100	—
2,5-[b]	—	98	+
Aniline, 2,4,6-trimethyl	88-05-1	98, 100	−
Aniline, ethyl			
2-	578-54-1	98, 100	−
3-	587-02-0	98, 100	−
Polycyclic fused aromatic hydrocarbons			
Acenaphthene	83-32-9	98, 100	+
Anthracene	120-12-7	98, 100	−
Benz[a]anthracene	56-55-3	100	+
Benz[a]anthracene, 7,12-dimethyl	57-97-6	100	+
Benzo[a]fluorene	238-84-6	100	+
Benzo[a]pyrene	50-32-8	98	+
Chrysene	218-01-9	100	+
Fluoranthene	206-44-0	98	+
Naphthalene	91-20-3	98, 100	−
Phenanthrene	85-01-8	98	+
Pyrene	129-00-0	1537	+
Triphenylene	217-59-4	98	+
1,2,3,4-Dibenzanthracene[b]	—	100	+
1,2,5,6-Dibenzanthracene[b]	—	100	+
Polycyclic fused heterocycles			
Acridine	260-94-6	1537	+
Carbazole	86-74-8	100	−
Isoquinoline[b]	—	98, 100	−
Quinoline	91-22-5	98	+
Quinoline, methyl			
7-[b]	—	100	+
8-[b]	—	100	+
Quinoline, dimethyl			
2,6-[b]	—	98, 100	−
Quinoline, hydroxy			
8-(sulfate)[b]	134-31-6	100	+
Quinoline, nitro			
8-[b]	607-35-2	100	+
Quinoline, amino			
8-[b]	578-66-5	1537	+
α-Naphthylamine[b]	—	100	+
2-Acetamidofluorene	53-96-3	98	+

[a] Data of Beauchamp and Shelby, (*15*). Environmental Mutagen Information Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

[b] Compounds not identified in shale oil, but predicted to occur in the same or modified chemical form.

mutagenic activity is a function of assay in a narrow concentration range; thus, toxic effects could override any genetic assay attempted, and components present at low concentrations might never be seen. Additionally, the choice of bacterial strain or the inducer involved in metabolic activation could be faulty for specific components. Furthermore, no one short-term test should be relied on for testing. Other systems (*17*) might complement one another. Metal ions might not be detected routinely in any of the short-term systems.

However, in the context of a prescreen for mutagenesis, and perhaps for carcinogenesis, the testing of crude mixtures with the Ames system is a feasible approach provided that appropriate fractionation, chemical analyses, and validation accompany the bioassays. A more important use of the short-term mutagenicity tests may lie in the dissection of a known response in a crude material and the tracing of the effect to the ultimate organic component(s) responsible for the potential damage.

The authors thank the staff at the Laramie Energy Research Center for their cooperation in providing samples and commenting on the research. We are indebted to Ms. Jennifer Young, Ms. Alice Hardigree, and Mr. Ira Rubin for the bulk of the technical work involved, and to Dr. Josef J. Schmidt-Collerus of Denver Research Institute for his encouragement throughout the course of these studies. We also thank the staff of the Environmental Mutagen Information Center for their help.

This research was jointly sponsored by the Environmental Protection Agency (IAG-D5-E681) and the Energy Research and Development Administration under contract with Union Carbide Corporation.

REFERENCES

1. Epler, J. L. Synfuel utilization: Environmental and health effects. In: Proceedings of the First Symposium on Management of Residues from Synthetic Fuels Production, Denver, Colorado, May 25-27, 1976. J. J. Schmidt-Collerus and F. S. Bonomo, Eds., ABC Reproductions, Denver, Colorado, 1976, pp. 314-319.
2. McCann, J., Choi, E., Yamasaki, E., and Ames, B. N. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals, Part I. Proc. Natl. Acad. Sci. (U. S.) 72: 5135 (1975).
3. Ames, B. N., McCann, J., and Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. 31: 347 (1975).
4. Rao, T. K., Rubin, I. B., Guerin, M. R., and Epler, J. L. Environmental mutagenesis of energy-related effluents. Genetics 83: s60 (1976).
5. Epler, J. L., Hardigree, A. A., Young, J. A., and Rao, T. K. Feasibility of application of mutagenicity testing to aqueous environmental effluents. Mutat. Res. 53: 88 (1977).
6. Rubin, I. B., Guerin, M. R., Hardigree, A. A., and Epler, J. L. Fractionation of synthetic crude oils from coal for biological testing. Environ. Res. 12: 358 (1976).
7. Epler, J. L., Young, J. A., Hardigree, A. A., Rao, T. K., Guerin, M. R., Rubin, I. B., Ho, C.-H., and Clark, B. R. Analytical and biological analyses of test materials from the synthetic fuel technologies. I. Mutagenicity of crude oils determined by the *Salmonella typhimurium*/microsomal activation system. Mutat. Res. 57: 265 (1978).
8. Rao, T. K., Young, J. A., Hardigree, A. A., and Epler, J. L. Analytical and biological analyses of test materials from the synthetic fuel technologies. II. Extended genetic and biochemical studies with mutagenic fractions. Mutat. Res. 54: 185 (1978).
9. Swain, A. P., Cooper, J. E., and Stedman, R. L. Large scale fractionation of cigarette smoke condensate for chemical and biologic investigations. Cancer Res. 29: 579 (1969).
10. Bell, J. H., Ireland, S., and Spears, A. W. Identification of aromatic ketones in cigarette smoke condensate. Anal. Chem. 41: 310 (1969).
11. Ho, C.-H., Clark, B. R., and Guerin, M. R. Direct analysis of organic compounds in aqueous by-products from fossil fuel conversion processes: Oil shale retorting, synthane coal gasification and COED coal liquefaction. J. Environ. Sci. Health, All (7): 481 (1976).
12. Guerin, M. R., and Epler, J. L. Determining emissions measurements needs for an emerging industry-advanced fossil fuels utilization. Paper presented at the First Conference on Determining Fugitive Emissions Measurements Needs, Hartford, Connecticut, May 17-19, 1976.
13. Bock, F. G., Swain, A. P., and Stedman, R. L. Bioassay of major fractions of cigarette smoke condensate by an accelerated technic. Cancer Res. 29: 584 (1969).
14. Patel, A. R., Haq, M. Z., Innerarity, C. L., Innerarity, L. T., and Weissgraber, K. Fractionation studies of smoke condensate samples from Kentucky Reference Cigarettes. Tob. Sci. 18: 58 (1974).
15. Beauchamp, R. O., and Shelby, M. D. Chemicals identified in oil shale and shale oil—preliminary list. Environmental Mutagen Information Center, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830.
16. Drake, J. W., Abrahamson, S., Crow, J. F., Hollaender, A., Lederberg, S., Legator, M. S., Neel, J. V., Shaw, M. W., Sutton, H. E., von Borstel, R. C., and Zimmering, S. (Committee 17). Environmental mutagenic hazards. Science 187: 503 (1975).
17. de Serres, F. J. The utility of short-term tests for mutagenicity. Mutat. Res. 38: 1 (1976).